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A RAPID AND HIGH-RESOLUTION METHOD TO DETERMINE THE COMPOSITION OF CORN SYRUPS BY LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic procedure was developed that provides saccharide distributions up to the pentasaccharides. The method requires less than 20 min and has been employed in research and in industrial laboratories. The precision and accuracy of results compare favorably with accepted but more time-consuming techniques.

INTRODUCTION

The high-performance liquid chromatographic (HPLC) separation of saccharides in sucrose, glucose and fructose mixtures is well known. Brobst *et al.*[1] and others[2–4] used cation-exchange resins with metallic counterions (Ca^{2+} and others) to separate the mono- and oligosaccharides in several carbohydrate sweeteners. Kesler[5] and Lee[6] used anion resins and a borate buffer eluent for their work. More recently, silica columns with specific functionalities have been used by Conrad and Fallick[7], Cegla and Bell[8] and Richter and Woelk[3] for sugar mixtures.

Several of the above procedures are excellent for separations up to the octasaccharides, but are either time-consuming[1,4] or require the use of undesirable organic solvents[3,7,8]. An alternative method[2,4] based on an aqueous eluent is rapid, but is limited to a molecular weight distribution up to the trisaccharides. Some procedures[5,6] provide excellent separations among the mono-, di-, and trisaccharides but are time-consuming and use systems of buffers as eluents. Fig. 1A, B and C are examples of excellent but time-consuming chromatograms; Fig. 1D is an example of a chromatogram obtained rapidly at the expense of the degree of separation.

In the work of Brobst and co-workers[1,4] with cation-exchange resins, many data were presented on the relative value of resin cross-linking but the discussion was limited to 4 and 8% cross-linked products. Resin particle size was also examined, but only when more resolution was needed among the tri- and lower saccharides to enable shorter analysis time with 8% cross-linked resin. Although not specifically mentioned, a separation based on an aqueous mobile phase was advantageous from economic, health hazard and waste disposal standpoints. It was clear that the benefits of this chromatographic approach—employing calcium counter-

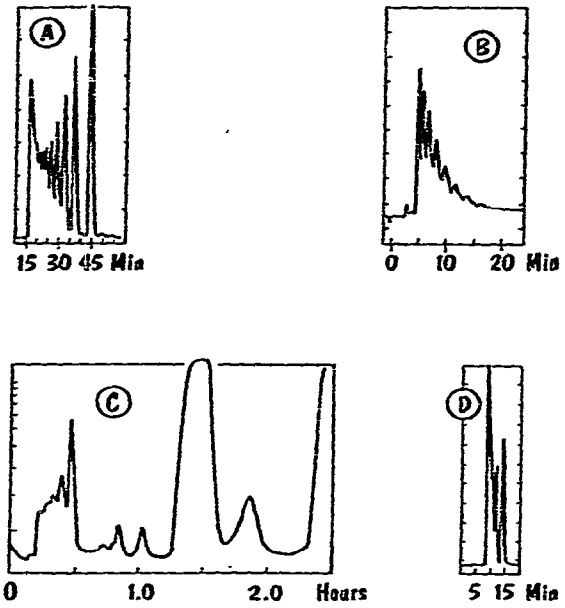

Fig. 1. A, Chromatogram of 42 D.E.* acid-hydrolyzed corn syrup, using a stainless-steel column (61 cm × 7 mm I.D.) packed with Aminex 50W-X4 (Ca^{2+}) and operated at 80°. The eluent was pumped at 0.5 ml/min, and a differential refractometer was used at ambient temperature. B, as in A, but using a column (30 cm × 3 mm I.D.) packed with chemically modified silica (μBondapak/CHO), and acetonitrile–water (60:40) as eluent at 1.0 ml/min. C, Partial chromatogram of a high-fructose corn syrup using a glass column (35 cm × 6.3 mm I.D.) filled with Durrum DA-X4 anion resin (borate) and operated at 55°. Borate buffer gradient (70 ml of 0.4% borate, pH 8.0, and 70 ml of 0.4% borate, pH 10.0) was pumped at 0.75 ml/min. An anthrone–sulfuric acid AutoAnalyzer detector was used and absorbance was plotted. D, Chromatogram of a 42 D.E. acid-hydrolyzed corn syrup using a stainless-steel column (61 cm × 7 mm I.D.) filled with Aminex Q-15S (8% cross-linked, Ca^{2+}) and operated at 80°. A differential refractometer was used at ambient temperature.

ions and aqueous eluents— could be further exploited by investigating the effect of resin cross-linking on both separation and speed of analysis. Perhaps a 4, 6 or 7% cross-linked cation-exchange resin of small particle size and with an appropriate metallic ion would provide a rapid oligosaccharide separation suitable for routine laboratory use. The objective was to develop a method with an analysis time under 20 min and providing separation up to the pentasaccharides. The result was, indeed, an HPLC determination of oligosaccharides up to the pentasaccharides that utilizes a controlled particle size, 4% cross-linked cation-exchange resin (Ca^{2+}). Approximately 17 min are needed for sample elution through glucose using water as eluent. An automatic sampler and integrator permit rapid processing of a large number of samples.

EXPERIMENTAL

Materials

A piece (30.5 cm × 7 mm I.D.) of chromatography grade stainless-steel tubing

* Throughout this article, D.E. (dextrose equivalent) is defined as a measure of reducing power using a modified Lane and Eynon procedure[12].

(Anspec., Ann Arbor, Mich., U.S.A.) was fitted with two 3/8-in. end fittings (Waters Assoc., Milford, Mass., U.S.A.), nuts and ferrules. Fittings with a 10-μm frit were selected for compatability with resin particle sizes. Strong cation resin was evaluated from three sources: A, Aminex 50W-X4, particle size 20–30 μm (Cat. No. 147-4208; Bio-Rad Labs., Richmond, Calif., U.S.A.), B, HC-X4.00 and HC-X6.00, particle size 10–15 μm (Cat. Nos. 77761 and 77768; Hamilton, Reno, Nev., U.S.A.) and C, BC-X4.00, particle size 10–15 μm (Cat. No. 605; Benson, Reno, Nev., U.S.A.).

Distilled deionized water from a Millipore Milli-Q water purification system (Millipore, Bedford, Mass., U.S.A.) was used in the preparation of aqueous solutions and as the mobile phase during the evaluation and analyses.

Apparatus

A Waters Model ALC 201 liquid chromatograph equipped with a Model 401 differential refractometer (capable of measuring 10^{-7} refractive index units) and a Model U6K universal injector was used throughout this study (Waters Assoc.). During the latter part of this investigation, a DuPont Model 834 (DuPont, Wilmington, Del., U.S.A.) automatic sampler was employed to provide 24-h operation during routine laboratory tests. A Spectra-Physics System I electronic integrator (Spectra-Physics, Santa Clara, Calif., U.S.A.) was used for peak integration and the calculations of results. Chromatograms were recorded on a Houston Omniscribe™ strip chart recorder (Houston Instruments, Austin, Texas, U.S.A.). Finally, an electrically heated column block of our own design (D. E. Just, CPC International) was used to maintain column temperature during the packing and evaluation of the analytical columns. It should be noted that stability of the detector is excellent but is dependent on ambient temperature if the temperature is not controlled with an external water-bath.

Resin preparation

The resin preparation method described by Brobst *et al.*[1] was used to clean the resin and convert it into the appropriate metallic form (*i.e.*, Ca^{2+}). Special attention, in the form of a double treatment, was given to the resin during the conversion step to insure complete metallic ion saturation and prevent sucrose inversion after the resin was packed. Resin fines, a detrimental fraction in some resin products, was not found to any appreciable amounts in the resin products tested.

Column packing

Analytical columns were packed using conventional slurry packing techniques. Briefly, two columns of the same length were connected using a tubing union. A column end fitting was attached to the bottom of the assembly and the lower section was inserted into a constant temperature block previously set to 80°. After securing the assembly into a vertical position, the column was filled with resin slurry, capped with an end fitting and pumped for several hours at *ca.* 0.1 ml/min above the final operating flow-rate. Then the flow was stopped, the top end fitting was removed and the resin bed depth was checked with a small probe. When more resin was required, the supernatant liquid was removed with a syringe attached to a narrow bore Teflon™ tube, more resin slurry was added, the end fitting was replaced and the pumping continued for another hour.

The resin bed was again checked for height and the above procedure was repeated as required. When the resin bed extended into the tubing union or above, the column was capped. Capping was accomplished by first removing the upper half of the column assembly and tubing union and immediately attaching a column end fitting, previously packed with *ca.* 3 mm of resin bed. In principle, the column could be used in either direction but the end capped last was usually designated as the inlet.

Sample preparation

Samples were adjusted to a *ca.* 5–10% solids basis. Particulates were removed by filtration through a 0.45-μm membrane filter. Soluble protein and inorganic salts were removed by ion-exchange. Samples with low pH can be quite harmful to the resin since localized concentration of hydrogen ions will displace the calcium ions and decrease the efficiency of the analytical column. The ease of sample preparation is one of the strengths of this and other similar procedures[4].

New column evaluation

A corn syrup conventionally prepared by acid hydrolysis (so-called acid-converted corn syrup) is a sample type well suited for column evaluation. Twenty microliters of a 10% solution offers the chromatographer a dual benefit. First, the number of theoretical plates can be calculated from the glucose peak, which is last to emerge. It is important that chart speed is adjusted to obtain desirable chromatograms so that small errors in measurement do not result in a large error in plate number. Second, the oligosaccharides possessing similar linkages and derived from a common monomeric form (*i.e.*, glucose) can be counted upward from glucose to determine the highest saccharide resolved. If there is doubt as to a particular saccharide assignment, assistance can be provided by constructing a plot of log molecular weight of saccharide *versus* elution time or volume as in Fig. 2. It is worthy of note that a plot of the saccharide molecular weights *versus* time or volume conforms to the

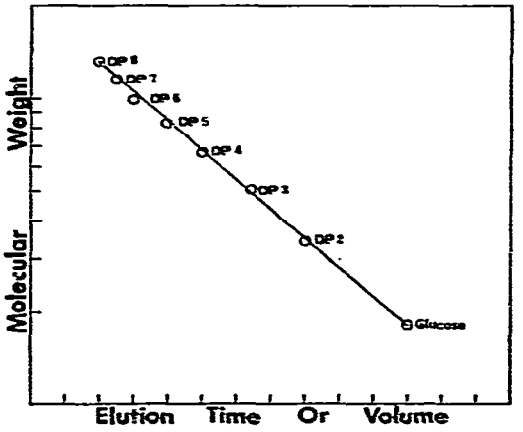

Fig. 2. Semi-log plot of molecular weight *versus* elution time or volume of the glucose homologous series. Saccharides were separated using a column (61 cm × 7 mm I.D.) of Aminex 50W-X4 ion-exchange resin (Ca^{2+}) operated at 80°. Water was pumped at 0.5 ml/min for elution and a differential refractometer used for the detector.

principle set forth by molecular exclusion chromatography. A periodic check of calcium saturation in the column can be accomplished using a dilute sucrose solution, since inversion of sucrose occurs when calcium is replaced by hydrogen ions on the resin[9].

RESULTS AND DISCUSSION

It is well known that small particles contribute to resolution within a given type of column packing, and that with the additional resolution comes an increase of pressure drop across the column. With the more highly cross-linked resins (6–8%) a high-pressure drop is not as damaging to resin bead integrity as with 4% cross-linked resins. With this in mind, 6 and 7% cross-linked resins (Ca^{2+}) of particle size 10–15 μm were tested in a (61 cm) column at 1.0 ml/min using water as eluent. It was hoped that, under these conditions, the 6 or 7% resin would combine the best

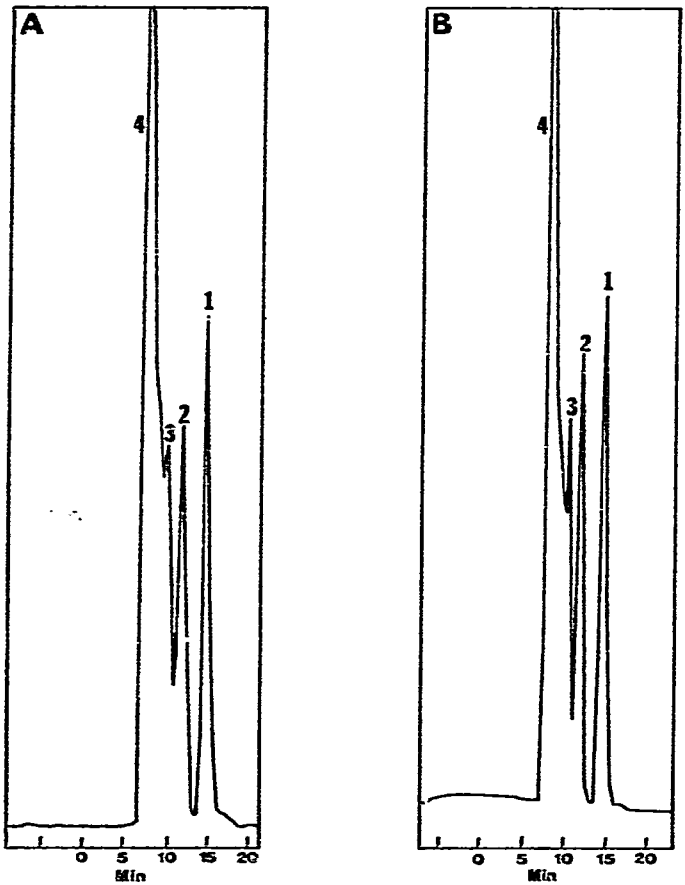

Fig. 3. Chromatograms of 42 D.E. acid-hydrolyzed corn syrup. Experimental parameters: column (61 cm × 7 mm I.D.) temperature, 80°; counterion, Ca^{2+}; flow-rate, 1.0 ml/min (water); column loading held essentially constant. Resin cross-linking: A, 8%; B, 6%. Peaks: 1 = glucose; 2 = maltose; 3 = maltotriose; and 4 = tetra- and higher saccharides.

features of the 4 and 8% cation resins. However, Fig. 3 shows a high degree of similarity between the 6 and 8% resin chromatograms: whereas a small particle size (7–10 μm), 6% cross-linked resin provided an acceptable value for the trisaccharides, it was not substantially better than an 8% resin and was not adequate for corn syrup characterization. In summary, the 6, 7 and 8% cross-linked ion-exchange resins (Ca^{2+}) were nearly equivalent in saccharide resolution, and the 4% crosslinked resin (Ca^{2+}) was still the best resolving medium.

The HC-X4.00 resin was available in a variety of particle sizes that might provide a better opportunity to optimize a separation, but primary emphasis was given to the particle sizes of 10–15 μm. All of the 4% resin tested was initially evaluated in a 61-cm column even though it was known that the chromatogram would be too time-consuming. The information obtained from the 61-cm column was used as a basis of comparison for the resins and also a means to relate to the prior work. Column pressure drop was found to be comparable for the resins tested. Resolution, as defined by the depth of the valleys between the peaks (peak sharpness) and the number of oligosaccharides resolved, was slightly better for the HC-X4.00 (particle size 10–15 μm) than the Aminex 50W-X4 (20–30 μm). The HC-X4.00 (particle size 7–10 μm) was examined briefly and gave a dramatic improvement to the overall resolution but it is not known how long the resin would perform at the higher pressure drops found. Because the 61-cm column procedures were too lengthy, mainly on account of the low flow-rates needed to maintain resin integrity, it was felt that a column [30.5 cm (1 ft.) × 7 mm I.D. (3/8 in. O.D.)] filled with a typical 4% resin and operated at 0.7 ml/min and at 80° could provide a desired analysis of corn syrup in the short time required.

Syrups examined

Fig. 4A, B, C and D show a variety of common corn syrups chosen because of the wide economic importance and for the differences in the saccharide distributions. Saccharide data from each of the various syrup types shown were quantitated on an area percent basis (component area expressed as a percent of the total area). Two reference procedures were used to analyze the syrups so that the results from the rapid method (described here) could be compared to the results from accepted methods. The reference methods were liquid chromatography[10] on high-resolution HC-X4.00 resin in a 2-ft. column pumped at 0.6 ml/min using saccharide calibration up to the trisaccharides, and classical quantitative paper chromatography[11]. Tables I–IV show the data comparison.

By virtue of the excellent agreement among the methods, the utility of the 30.5 cm column procedure was demonstrated. It was felt, however, that precision was equally important. Precision, expressed as the coefficient of variation, CV, can be demonstrated in several different ways. The same day and day-to-day variations were calculated for a typical syrup on a research basis during the method development. Because the procedure is rapid (less than 20 min), the approach lends itself to the needs of routine laboratory use. To test the utility of the procedure in a laboratory environment, an automatic sampler was added for 24-h operation. As a part of the normal operation, a daily control sample analyzed with other samples served two important purposes: (1) to monitor daily the quality of performance of the system by observing separation and quantitation; (2) to observe the long term

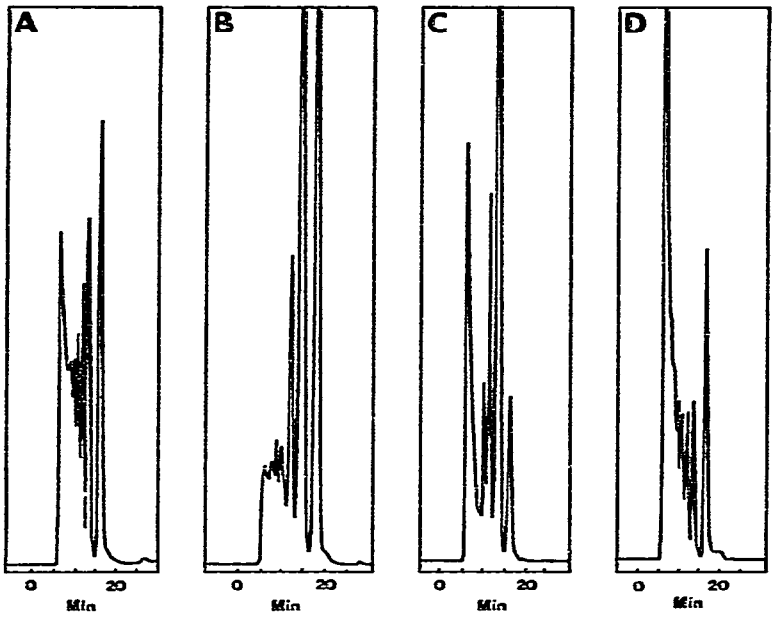

Fig. 4. Chromatograms of a 42 D.E. acid-hydrolyzed corn syrup (A), a 62 D.E. enzyme-converted corn syrup (B), a 42 D.E. high-maltose syrup (C) and a 30 D.E. acid-hydrolyzed corn syrup (D). Chromatographic conditions: stainless-steel column (30.5 cm × 7 mm I.D.) packed with Aminex 50W-X4 (20–30 μm) operated at 80° and pumped with water using a flow of 0.6 ml/min.

TABLE I

COMPARISON OF COMPOSITION BY LIQUID AND PAPER CHROMATOGRAPHY FOR 42 D.E. ACID-CONVERTED CORN SYRUP

*Component identification**	*% by liquid chromatography*		*% by paper chromatography*
	*1-ft.***	*2-ft.****	
DP 1	19.3	19.2	19.3
DP 2	14.1	13.8	14.4
DP 3	11.7	11.3	12.0
DP 4	9.3	9.0	9.9
DP 5	7.8	7.6	8.0
DP 6+	37.6	39.0	35.8

* Oligosaccharide degree of polymerization.

** Aminex 50W-X4 resin (20–30 μm) operated in a 30.5-cm (1-ft.) column at 80° using water at a flow-rate of 0.6 ml/min.

*** Aminex 50W-X4 resin operated in a 61-cm column at 80° using water at a flow-rate of 0.6 ml/min.

performance during the life of the analytical column. Table V shows typical values for precision.

The first column evaluated operated for about 2 months during which time about 1400 samples were analyzed. During this period, values for monosaccharides up to the pentasaccharide were provided although slight corrections were applied to the

area percent calculations near the end of the life of the column. A 2-month column-life is considered average for our application. This value could vary widely depending on the quality of samples injected, quality of eluent and general operating procedures.

TABLE II

COMPARISON OF COMPOSITION BY LIQUID AND PAPER CHROMATOGRAPHY FOR A 62 D.E. ENZYME-CONVERTED CORN SYRUP

Details as in Table I.

Component identification	*% by liquid chromatography*		*% by paper chromatography*
	1-ft.	*2-ft.*	
DP 1	46.7	46.2	47.4
DP 2	27.1	27.2	26.8
DP 3	6.2	6.1	6.1
DP 4	5.3	5.1	4.5
DP 5	3.8	3.6	3.6
DP 6^+	10.9	11.8	11.2

TABLE III

COMPARISON OF COMPOSITION BY LIQUID CHROMATOGRAPHY AND PAPER CHROMATOGRAPHY FOR A HIGH MALTOSE SYRUP

Because of the saccharide distribution, only DP 5^+ is reported instead of DP 6^+. Other details as in Table I.

Component identification	*% by liquid chromatography*		*% by paper chromatography*
	2-ft.	*1-ft.*	
DP 1	7.1	7.5	7.5
DP 2	39.3	39.8	40.1
DP 3	15.4	15.1	15.3
DP 4	7.3	7.1	7.8
DP 5^+	30.9	30.5	29.3

TABLE IV

COMPARISON OF COMPOSITION DATA FROM TWO METHODS OF LIQUID CHROMATOGRAPHY FOR A 30 D.E. ACID-CONVERTED CORN SYRUP

Details as in Table I.

Component identification	*% by liquid chromatography*	
	2-ft.	*1-ft.*
DP 1	14.4	14.4
DP 2	7.1	7 0
DP 3	6 3	6.4
DP 4	5.9	6.1
DP 5	6.0	6.1
DP 6^+	60.1	60.0

TABLE V

ANALYTICAL PRECISION FOR CORN SYRUPS USING THE PROPOSED RAPID PROCEDURE

Component	*Coefficient of variation*			
	*Research data (short term)**		*Routine lab. data (long term)***	
	Product A	*Product B*	*Product A*	*Product B*
DP 1	0.41	0.20	1.55	1.06
DP 2	0.21	0.33	2.03	1.25
DP 3	0.60	0.97	2.18	2.44
DP 4	0.22	1.13	3.80	5.14
DP 5	0.77	0.79	6.46	6.28
DP 6+	0.48	1.01	2.25	2.49

* Measured over the span of several days.
** Measured over the span of 6–8 weeks.

CONCLUSION

We conclude that a quality 4% resin packed in a column (30.5 cm × 7 mm I.D.) operated at 80° using water as the eluent pumped at 0.6 ml/min offers a rapid (less than 20 min), accurate means for saccharide characterization of corn syrup. With peripheral equipment, such as an automatic sampler and computing integrator, this system is a powerful tool for routine laboratory analysis.

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